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TREATMENT OF ASTHMA

INS A1

This invention relates to the treatment of asthma. More particularly, it relates to both therapeutic treatment of asthma sufferers and to preventative (prophylactic) treatment of non-asthmatics against asthma.

BACKGROUND ART

Asthma is a chronic inflammatory disorder of the airways in which many cells play a role, including mast cells and eosinophils. In susceptible individuals this inflammation causes symptoms which are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment, and causes an associated increase in airway responsiveness to a variety of stimuli.

Asthma can be inherited, is not contagious and may be chronic and persistent or occurring in the form of attacks which are periodic and usually at least partly reversible. Attacks vary in severity and frequency from person to person. Many factors may contribute to the development of asthma including exposure to inhaled allergens such as pollens, mold spores, house dust mites and animal dander. In an individual who has developed asthma, many stimuli can trigger asthma attacks including allergens, viral respiratory infections (colds or the flu), irritants in the air (smoke, air pollution, perfume), damp, cold weather, and exercise.

During an asthma attack, the muscles around the bronchial tubes tighten and the linings of the bronchial tubes swell (become inflamed) and produce thick mucus, thereby decreasing the internal diameter of the tubes. These changes increase resistance to the flow of air making it hard to breathe. When asthma is properly controlled the bronchial tubes are of normal size.

Asthma is a common disease among both children and adults. An estimated 7% of people in the United States have been diagnosed as asthmatic. The corresponding figure for New Zealand is about 10% (Burney, P. *et al.* (1996) Variations in the Prevalence of Respiratory Symptoms, Self-Reported Asthma Attacks, and Use of Asthma Medication in the European Community Respiratory Health Survey. *Eur. Respir. J.* 9:687-695). The occurrence of asthma in both Western and developing

countries has increased markedly over the last 30 years. This relatively short time frame suggests that environmental rather than genetic factors are at work.

In most cases asthma is an atopic disorder in which the underlying process is due to an allergic response to common environmental allergens. This allergic response is a function of the immune system characterised by activation and recruitment of eosinophils to the lung causing the characteristic chronic swelling and inflammation of the airways that affects the breathing of sufferers.

The pharmaceutical treatment of asthma includes several different classes of drugs, including beta agonists, topical or oral steroids and theophyllines. If used appropriately, such treatments may keep asthma systems from developing or relieve them when they are present. Beta agonists and theophyllines primarily act by relaxing the muscles surrounding the airways while steroids act to reduce (and even prevent) inflammation and mucus production. Other medications exist and more are being developed due to the growing interest in and concern over the prevalence, morbidity and mortality of asthma world-wide.

There is an immunological basis to the development of airways inflammation in asthma, involving the Th2 lymphocytes (Th2s). These cells secrete cytokines, including interleukin-4 (IL-4) and IL-5, leading to enhanced production of immunoglobulin E (IgE) by B cells and the generation and recruitment of eosinophils respectively. Activation of mast cells by allergens releases histamine and other mediating chemicals that trigger an acute inflammatory response, including mucus production. Eosinophils release mediators including cytotoxins which lead to inflammation and necrosis of the bronchial epithelium. The localised recruitment and activation of eosinophils together with the resultant tissue damage is termed "eosinophilia".

A need therefore exists for an asthma treatment that modulates the immune system to reduce the risk of developing atopy and airways inflammation, in addition to the traditional treatment with drugs which suppress airways inflammation once it has already occurred, or drugs which reduce symptoms in an asthmatic individual. An added benefit would be if such a treatment also has a similar inhibitory effect in a current sufferer of an atopic disorder to reduce the severity of their disease.

One immunological approach to meet this need involves *Mycobacterium bovis - Bacillus Calmette-Guerin* (BCG). Prior active infection with this organism has been reported by Erb *et al* (*J. Exp. Med.*, Vol. 187, No. 4, February 16 1998) to suppress subsequent allergen-induced airway eosinophilia in mice, with intranasal infection being reported to be more effective than intraperitoneal or subcutaneous infection.

BCG as an organism and as BCG-Polysaccharide Nucleic Acid has also been reported as being used in the treatment of asthma in China (see, for example, *China J. Paedia* (1991); 39(3): 165-167, *Guangzhou Medical Journal* 1984; 15(2):16-18) and *Acta of Hu-Nan Medical University* 1992; 17:365-367. Intact BCG is reported as being administered both alive and dead. The reported routes of administration vary between intramuscular injection and scratch vaccination.

The present invention is directed to an alternative immunological approach involving, as active agent, a type of lipopolysaccharide in immunogenic form. The lipopolysaccharide is lipoaribinomannan (LAM).

Lipopolysaccharides (including LAM) have been included in immunological compositions previously. For example, US Patent specification 5,853,737 (Modlin) discusses various methods of inducing a CD1 restricted immune response and teaches of a vaccine containing CD1-presented non-polypeptide hydrophobic antigens and in particular a lipoarabinomannan (LAM) antigen.

Both US Patent specifications 4,329,452 and 4,394,502 (Maruyama) teach of the use of lipopolysaccharide as an active component in an immunotherapeutic agent for tumours. The lipopolysaccharide can be derived from human tubercle bacillus.

However, to the applicant's knowledge, LAM has not been employed as an immunoactive agent in a vaccine for treating asthma, either prophylactically or therapeutically, where the vaccine is administered to the airways of a patient.

It is therefore an object of this invention to provide an immunological approach to the treatment of asthma, both prophylactically (in relation to non-asthmatics) and therapeutically (in relation to asthmatics) which at least provides a useful choice over existing approaches.

SUMMARY OF THE INVENTION

In a first aspect, the invention provides a vaccine for inducing an immune response in a patient effective in the prophylactic treatment against, or therapeutic treatment of, asthma which comprises, as active agent, immunogenic lipoarabinomannan (LAM) formulated for respiratory administration to said patient.

As used herein, "immunogenic LAM" means LAM other than as part of an intact mycobacterial organism, which LAM is capable of inducing an immune response in a patient.

Preferably, said immunogenic LAM is substantially free of bacterial nucleic acid.

As used herein, "prophylactic treatment against asthma" means treatment of a non-asthmatic patient to prevent or at least reduce the likelihood of the patient becoming asthmatic.

As used herein, "therapeutic treatment of asthma" encompasses preventing, or reducing the severity of the symptoms of an asthmatic episode in an asthmatic patient, inclusive of bronchial inflammation and eosinophilia.

As used herein, "respiratory administration" means administration to the airways of a patient, including administration intranasally and by inhalation through the mouth to reach the respiratory tract.

The immune response induced is a non-CD1 restricted immune response.

The invention further provides a vaccine for reducing the severity of asthma comprising an immunologically effective amount of immunogenic LAM formulated for respiratory administration.

Still further, the invention provides a vaccine for reducing the risk of developing asthma comprising an immunologically effective amount of immunogenic LAM formulated for respiratory administration.

Conveniently, said immunogenic LAM is isolated from a mycobacterium, more conveniently isolated from an *M. bovis* organism and most conveniently is isolated from *M. bovis* strain AN5.

Preferably, said LAM contains, as its saccharide component, from 27% to 52% mannose and from 73% to 48% arabinose, more preferably, from 40% to 50% mannose and from 60% to 50% arabinose, and most preferably approximately 45% mannose and approximately 55% arabinose.

It will be usual for said immunogenic LAM to be a fluid, and preferably in the form of a solution or suspension.

Conveniently, the vaccine will further comprise a respiratorially acceptable adjuvant, which may include a detergent or surfactant component.

A secondary immunogen selected from one or more Th1 type immune response inducing substances may also be present. Preferably, *Mycobacterium bovis* (Bacillus Calmette-Guerin) is included as said Th1 type immune response inducing substance.

In another aspect, the invention provides a method of prophylactically treating a non-asthmatic patient against asthma which comprises the step of inducing an immune response in said patient by respiratorially administering an effective amount of immunogenic LAM.

In yet another aspect, the invention provides a method of therapeutically treating asthma in a patient which comprises the step of inducing an immune response in said patient by respiratorially administering an effective amount of immunogenic LAM.

Again, the immune response induced is not a CD1 restricted immune response.

Conveniently, said immunogenic LAM is administered in the form of a vaccine as described above.

Usually, the immunogenic LAM will be administered by inhalation through the mouth or intranasally to said patient.

In yet another aspect, the invention provides the use of immunogenic LAM in the preparation of a medicament for the therapeutic treatment of asthma.

In still another aspect, the invention provides the use of immunogenic LAM in the preparation of a medicament for prophylactic treatment of a non-asthmatic against developing asthma.

In preferred embodiments, the immunogenic LAM is isolated from a mycobacterium, more preferably an *M. bovis* organism, and most preferably *M. bovis* strain AN5.

It is further preferred that the immunogenic LAM contains, as its saccharide component, from 27% to 52% mannose and from 73% to 48% arabinose, more preferably from 40% to 50% mannose and from 60% to 50% arabinose and most preferably approximately 45% mannose and approximately 55% arabinose.

It will be usual in preparing said medicament that said immunogenic LAM be combined with a respiratorially acceptable adjuvant such that the medicament is formulated for respiratory administration.

In a final aspect, the invention provides a device for prophylactically or therapeutically treating asthma which includes a container from which a vaccine as described above can be dispensed to the airways of a patient in need of such treatment.

The device will conveniently be one from which said vaccine is dispensable for inhalation through the mouth of a patient, or intranasally dispensable.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing number of cells recovered per ml of bronchoalveolar lavage (BAL) exudate.

Figure 2 is a graph showing total number of cells recovered by BAL.

Figure 3 is a graph showing the percentage of eosinophils recovered by BAL.

Figure 4 is a graph showing the percentage of macrophages recovered by BAL.

Figure 5 is a graph showing number of eosinophils recovered per ml of BAL exudate.

Figure 6 is a graph showing total number of eosinophils recovered by BAL.

Figure 7 is a graph showing the dose response curve for LAM as determined by numbers of eosinophils recovered per ml of BAL exudate.

Figure 8 is a graph showing the effect of LAM in CD1 Knock Out mice as determined by the number of eosinophils recovered per ml of BAL exudate.

BEST MODE OF PERFORMING THE INVENTION

As broadly outlined above, the present invention offers an approach to reducing the severity of airway eosinophilia and thus asthma in an asthmatic and/or for reducing the risk of developing airway eosinophilia and thus asthma in a non-asthmatic by introducing to the airways biologically active amounts of lipoarabinomannan (LAM) in an immunogenic form.

LAM is present in actinomycetes, which are a distinctive lineage of Gram-positive bacteria. Members of this lineage include *Rhodococcus equi, Corynebacterium diphtheriae, Corynebacterium matruchotii, Gordona rubropertincta, Gordona terrae, Rhodococcus rhodnii* and *Tsukamurella paurometabolum.*

Other members of the lineage include mycobacteria, with LAM being a major lipoglycan of the mycobacterial cell wall.

For use in the present invention, LAM can therefore be obtained by isolation from any suitable actinomycetes organism. It is however preferred that the immunogenic LAM for use in the invention be obtained from mycobacteria, particularly pathogenic mycobacteria, or from attenuated strains of pathogenic mycobacteria. However, LAM from non-pathogenic avirulent mycobacteria is by no means excluded.

The preference for pathogenic mycobacteria as a source for immunogen LAM arises from the association between LAM, mycobacterial pathogenesis and the ability of LAM to modulate Tumour Necrosis Factor-alpha (TNF-α) production (see the article "Relationships Between the Structure and the Roles of Lipoaribinomannans and Related Glycoconjugates", Vercellone *et al.*, *Frontiers in BioScience* 3, p 149-163 (1988)).

Particularly suitable mycobacteria from which LAM can be obtained are therefore *M. bovis*, *M. tuberculosis*, *M. vaccae* and *M. paratuberculosis*, with *M. bovis* organisms such as *M. bovis* strain AN5 being presently preferred.

The LAM can be isolated from such bacteria, and in particular from mycobacteria, using techniques which are standard in the art. By way of example, the procedure of Severn *et al.*, *J. Microb. Methods*, 28, 123-30 (1997) can be employed.

Isolated LAM will conveniently be purified for use in the present invention. The effect of this will be to exclude other bacterial components (including bacterial nucleic acid) from the LAM. Again, art standard techniques can be employed such as those described by Severn *et al.*

The saccharide composition of the immunogenic LAM can vary. Generally, any lipoglycan with a saccharide component containing both arabinose and mannose (and therefore qualifying as a LAM) can be used. However, it is preferred for there to be at least 27% of mannose present, with a preferred saccharide composition varying from 27% to 52% mannose and 73% to 48% arabinose.

More commonly, the saccharide component will include 40% to 50% mannose and 60% to 50% arabinose, with one particularly preferred LAM having a saccharide component which is approximately 45% mannose and 55% arabinose.

Once the LAM is obtained and preferably purified, it is formulated for respiratory administration. Respiratory administration requires delivery of the LAM to the airways of the patient to be treated. Generally, this will involve delivery through the mouth or intranasally. Often, inhalation by the patient will provide the motive force to the LAM. However, respiratory administration can also involve delivery by

propellant, including in the form of an aerosol generated using a jet or ultrasonic nebuliser. This is presently preferred.

For such applications, the LAM will conventionally be in a fluid form. This can be as a powder or as a solution or suspension (particularly for aerosol application).

The LAM will generally also be formulated for respiratory administration together with a respiratorially acceptable adjuvant. The selection of the adjuvant will be dependent upon the formulation and mode of dispensing involved, but will in any case be a matter of routine choice for the skilled worker in this field.

Where, as is preferred, the LAM is to be administered via a nebuliser-generated aerosol, the LAM will be in the form of a solution or suspension which will contain such adjuvant components. One such optional but preferred component is a non-toxic detergent or surfactant. Examples include a Polysorbate 80, beractant (Survanta Susp (Abbott)) and colfosceril palmitate (Exosurf Neonatal (Glaxo Wellcome)).

It is also possible to include an additional immunogen in the solution or suspension for administration as an aerosol. Such an immunogen will generally be a Th1 type immune response inducing substance. One such substance which can be included is BCG, alive or dead, but with dead being preferred.

Where BCG is included as a secondary immunogen, it will be usual for the solution or suspension to further comprise a non-clumping agent (such as Bovine Serum Albumin) to prevent the organisms from adhering together.

Despite the preference for aerosol administration, it is by no means intended to exclude administration of LAM in other forms. To the contrary, the LAM vaccine can be formulated for administration as a powder, for example using lactose capsules as a delivery vehicle in a dry powder inhaler.

The invention will now be exemplified through reference to the following experimental section, which it will be appreciated is illustrative and not limiting.

EXPERIMENTAL

SECTION A

LAM Isolation

Isolation of LAM from *M. bovis*

LAM was isolated and analysed by the procedures of Severn *et al.*, *J. Microb. Methods*, 28, 123-30 (1997) as described briefly below.

M. bovis strain AN5 (obtained from Central Veterinary Laboratories, Weybridge, UK) was grown as pellicles on modified Reid's synthetic medium. The cells were killed by heating at 100°C for 3 hours, washed with buffered saline and recovered by centrifugation. The cells were slurried in TBS, cooled and extruded by passing though a French pass. The disrupted cells were digested with RNAse (Boehringer Mannheim) and DNAse (Boehringer Mannheim) at 37°C and then 60°C.

Triton X-114 solution was added to the lysed cells, cooled on ice and stirred for 16 hours at 4°C. The cellular debris was removed by centrifugation and the supernatant was incubated at 37°C to induce phase separation. The lipoglycans were recovered from the lower Triton X-114 phase following precipitation by the addition of ethanol and centrifugation. The extract was further purified by treatment with Proteinase K and isolated by ultra-centrifugation. The lipoglycans were resolved into their separate species by size exclusion chromatography on Sephacryl s-200. Fractions containing LAM were identified using SDS-PAGE analysis.

Analysis of lipopolysaccharide

The purity of the combined LAM fractions was investigated. LAM was deemed pure based on the following criteria: 0% protein as indicated by the BCA protein assay performed as described by Severn *et al* (1997), absence of nitrogen as indicated by elemental analysis of the purified extracts, and the absence of ribose or deoxyribose in the glycose analysis (Severn *et al* (1997)).

The purified LAM was hydrolysed and acetylated (as described by Severn *et al* (1997)) and the resulting mixture of saccharides analysed by GLC as described by Severn *et*

al (1997)). The mixture was comprised of 45% mannose and 55% arabinose confirming that the saccharide component of the lipoglycan is arabomannin.

SECTION B

Formulation of LAM for respiratory administration

LAM, purified as in Section A, was formulated for intranasal administration in Phosphate Buffered Saline (PBS).

SECTION C

Efficacy of LAM

SECTION C1

Materials and Methods

An ovalbumin (OVA) induced airway eosinophilia mouse model of atopic airway inflammation was used to determine the effectiveness of the immunogenic LAM suppressing the development of airway eosinophilia. This model is widely used to establish "asthma-like effects" in mice - see for example, Erb *et al.*, *J. Exp. Med.* 187(4):561-569 (1998); Herz *et al.*, *J.Allergy and Clinical Immunology*, 102:867-874 (1998); and Randaolf *et al.*, *J. Clinical Investigation*, 104:1021-1029 (1999).

Briefly, antigen specific Th2 cells were primed to OVA in test mice by two successive intraperitoneal immunisations 14 days apart with OVA and by administration of an intranasal challenge of OVA 14 days after the second intraperitoneal immunisation.

To test efficacy of LAM, biologically active amounts of LAM in PBS were given intranasally to 5 primed mice 7 days after the second intraperitoneal immunisation. PBS was also administered intranasally to 5 primed mice as a control. 4 days post intranasal challenge bronchoalveolar lavage (BAL) was used to determine the degree of eosinophil inflammatory response in all mice. BAL exudates were examined for the presence of eosinophils.

BCG, alive or killed by heating at 56°C for 30 minutes, were given to groups of 5 mice at doses of 2×10^6 colony forming unit (CFU) equivalents for comparison purposes. The doses were administered intranasally and their effect determined by BAL as described above.

In each case, PBS was administered intranasally to 5 primed mice as a control.

3 dosages of LAM in PBS were administered to the airways of 5 primed mice 7 days before intranasal OVA challenge. The dosages were 12 μg/ml (high), 1.2 μg/ml (intermediate) and 0.12 μg/ml (low). The effect of LAM on airway eosinophilia was determined by BAL. Again, PBS was administered intranasally to 5 primed mice as a control.

Results and Discussion

Figures 1 to 6 show the results of these experiments. Figures 1 and 2 show the number of cells recovered per ml and in total. Figures 3, 5 and 6 show that immunisation with high dose LAM gives rise to a reduction in eosinophil numbers equal to or greater than the reduction seen with whole live or dead BCG. This implies that LAM may be the active component in BCG that suppresses airway eosinophilia. Figure 4 shows that the number of macrophages in mice immunised with whole BCG (live or dead) is equivalent to the number found in mice immunised with high doses of LAM. This is supportive of a finding that TNF-α (which activates macrophages) is stimulated by LAM.

<u>SECTION C2</u>

Materials and Methods

The OVA induced airway eosinophilia mouse model of atopic airway inflammation described in section C1 was used, together with a CD1 Knock-Out (KO) mouse model. The CD1 KO mice used were bred in turn from CD1 KO mice, prepared as described by Chen *et al.*, *Immunity*, 6:459-467 (1997), and confirmed as having CD1 KO status by standard techniques. Briefly, these involved FACS staining of the mice as follows:

Mice were tail bled and the cells spun down into a pellet. The cells were treated with ACK lysis buffer and then spun down into a pellet. The pellet was resuspended in FACS buffer (PBS + 2% foetal calf serum + 0.1% sodium azide). The cells were then stained with PE-anti-CD1d and analysed by flow cytometry to identify CD1 KO mice.

To establish a dose response curve for LAM, doses of 3.6 to 12 μg/ml of LAM obtained as in Section A and formulated as in Section B were administered intranasally to the airways of 5 OVA-primed mice 7 days prior to intranasal OVA challenge. PBS was administered intranasally to 5 primed mice as a control. The effect of LAM on airway eosinophilia was determined by BAL.

To establish the effect of LAM in CD1 KO mice, a dose of 12 μg/ml LAM obtained as in Section A and formulated as in Section B was administered intranasally to the airways of 5 CD1 KO mice 7 days prior to intranasal OVA challenge. PBS was administered intranasally to 5 CD1 KO mice as a control. The effect was determined by BAL.

Results

Figure 7 shows the effective dose of LAM with respect to a reduction in eosinophil numbers compared to a PBS control.

Figure 8 shows that immunisation with LAM gives rise to a reduction in eosinophil numbers in CD1 knockout mice. This shows that the LAM-induced reduction in eosinophil numbers is not dependent on the CD1 pathway, and the immune response is not CD1 restricted.

INDUSTRIAL APPLICATION

As will be appreciated from the above, the primary application of the invention is in anti-asthma treatment. That treatment may be prophylactic, to prevent or reduce the risk of non-asthmatics developing asthma, or therapeutic, to suppress established disease in an asthmatic.

The LAM-containing vaccines of the invention are formulated for respiratory administration, which will preferably involve the inhaled route for convenience. In turn, the presently preferred mode of administration will involve the use of a dispensing device, of which a container of LAM vaccine forms a part. That device can be a nebuliser, particularly a jet nebuliser such as that known as the Omron CX (Omron Healthcare, Singapore), the Medic Aid Ventstream or the Wright nebuliser (Aerosol Medicals, Colchester, UK) (where the vaccine is to be administered as an aerosol) or a dry powder inhalation device (such as the devices known as the Accuhaler and Diskhaler (Glaxo Wellcome)).

Respiratorially administered LAM has shown significant efficacy in reducing eosinophil numbers and in turn in reducing bronchial inflammation. The implications of this in both resisting the onset, and reducing the severity, of an asthma episode, and in treating non-asthmatics against developing asthma will be apparent to those skilled in this art.

Having described preferred methods of putting the invention into effect, it will be appreciated that modifications can be effected and yet still come within the general concept of the invention. It is to be understood that all such modifications are intended to be included within the scope of the present invention.